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# HtrA, RseP, and Tsp do not elicit a pathology related serum IgG response during sexually transmitted infection with *Chlamydia trachomatis*

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**Abstract**

*Chlamydia trachomatis* sexually transmitted infection can cause serious reproductive morbidities. This study determined the prevalence of serum IgG response to *C. trachomatis* putative stress response proteins in females to test for an association with genital tract pathology. There was no significant association of serum IgG to HtrA, Tsp, or RseP with infection or pathology. cHSP60 serum IgG prevalence was significantly associated with infection compared to negative (infertile) controls ($p = 0.002$), but not with upper genital tract pathology. Serum $IgG_{1-4}$ antibody subclasses reactive with the antigens was not significantly different between cohorts, although different responses to each antigen were detected.

## Introduction

*Chlamydia* (*C.*) *trachomatis* is the most common bacterial sexually transmitted infection world-wide. There is considerable reproductive morbidity resulting from infection. Serological markers have been widely considered as a potential indicator of female upper genital tract pathology due to *C. trachomatis* infection. Studies have largely focussed on the heat shock proteins (cHSP10 and cHSP60). However, not all studies support that serum antibodies to cHSPs are significantly associated with tubal factor infertility, with some studies showing a significant association at a high titre (Spandorfer et al., 1999, Persson et al., 1999), and other reports finding no significant association (Debattista et al., 2002).

The proteins included in our current study (HtrA, Tsp, RseP) were selected for their potential role as proteases during chlamydial stress response. Although since commencement of this project, Tsp has also been demonstrated to have an immune evasion role for *Chlamydia* (Lad et al., 2007). Research from our own team has also provided evidence of a stress response, and penicillin persistence role of HtrA (Huston et al., 2008, Huston et al., 2007). RseP function remains unknown. This study aimed to investigate whether these proteins and cHSP60, are serological markers of *C. trachomatis* sexually transmitted infection and upper reproductive tract pathology.

## 2. Materials and Methods

### *2.1 Collection of samples, analysis of patient histories and definition of cohorts*

Participants were sourced from the following Australian venues and serum collected (Human Research Ethics Approval Numbers are indicated in parentheses): Brisbane Sexual Health Clinic and Family Planning Queensland Fortitude Valley Clinic (EC2809), Nambour Sexual Health Clinic (EC2809); Ipswich and West Moreton Sexual Health Clinic (10-09); Gold Coast Sexual Health Clinic (200893); Cairns Sexual Health Clinic (HREC/09/QCH/4-554); and Wesley IVF and Gynaecology Clinic (2008/02). Queensland University of Technology Human Research Ethics approval was obtained for the study (0800000268). Consent was obtained individually from each participant and patient history, clinical data, and *Chlamydia* serology used to assign cohorts (Supplementary Data 1).

*2.2 Testing for serum antibodies to the protein candidates*

Western blots against purified recombinant proteins were used to assess the serological responses (Supplementary Data 1). Serum IgG to *C. pneumoniae* was tested in a random selection of 91 participants (from all cohorts) using a commercial kit (BioClone). Serum IgG to *C. trachomatis* was tested in all participants in the infertile and pathology cohorts (BioClone).

*2.3 Generation of the purified recombinant proteins*

The recombinant protein constructs for HtrA (Huston et al., 2007), and cHSP60 (Debattista et al., 2002) have been previously reported. The Tsp construct will be published

elsewhere, and the RseP recombinant expression construct was developed for this study (Supplementary Data 2).

*2.4 Statistical analysis*

All statistical analysis was conducted using the program SPSS. Significance of reactions with cohort grouping was tested using Fisher's exact chi Square test, with only p values $< 0.05$ considered to be significant.

## 3. Results

*3.1 HtrA, RseP and Tsp are antigens for serum IgG during* C. trachomatis *STI, but are not associated with pathology*

Serum from all participants was tested by western blot to detect IgG to each of the proteins, see Table 1. The lower genital tract infection (LGT), upper genital tract pathology (UGT), and multiply-infected (Multiple) cohorts all had confirmed histories of *Chlamydia* sexually transmitted infection (STI). The infertile cohort participants tested negative for *C. trachomatis* serology using a commercial kit and had no recorded clinical history of *C. trachomatis* or evidence of tubal damage. Serum IgG for *C. pneumoniae* was observed in 57 % of the participants from all cohorts.

The prevalence of IgG positive reactions to HtrA in serum from participants with *C. trachomatis* infection (LGT, UGT, and Multiple) were not significantly different from that observed for the Infertile cohort (Fisher's Exact Chi Square test, p = 0.233). HtrA total IgG positive reaction was similar within the infected cohorts (i.e. LGT, UGT, and Multiple only) and had no association with any cohort. Similarly, the prevalence of IgG to Tsp and RseP were not significantly associated with infection (Fisher's exact Chi square test Tsp, p= 0.585, RseP p=0.695), or pathology (UGT) (Tsp p= 0.659).

Serum IgG positive reactions for cHSP60 was significantly associated with infection when the infection cohorts were analysed compared to the infertile cohort (negative) by Fisher's Exact Chi square test (p = 0.002). There was no association of cHSP60 IgG positive reaction with the pathology (UGT) compared to uncomplicated infection (LGT) or multiple infection (M) cohorts when analysed by Fisher's Exact Chi square (p=0.888) (Table 1).

*3.2 Serum IgG to a combination of the proteins is not associated with pathological outcomes*

The prevalence of participants with serum IgG antibodies reactive to any combination of at least two of the antigens was not significantly associated with either infection, or pathology. There was a slight variation (not significant) in the prevalence of serum IgG to both HtrA and cHSP60 between the cohorts (24.5 % LGT, 35.3 % Multiple, and 33.3 % UGT; not significant). The prevalence of serum IgG to both Tsp and cHSP60 was slightly higher in participants with multiple infections (M) compared to those with a single infection (LGT) or a pathological outcome (UGT) (LGT; 13.2 %, M; 17.6 %, UGT; 11.1 %; not

significant). The prevalence of serum IgG to at least three of the antigens was analysed and found to be not significantly associated with any cohort.

*3.3 The different antigens produced different IgG subclass responses*

There were 58 participant samples in the study that had serum IgG antibodies against HtrA, 15 of which were detected to react with $IgG_{1-4}$ subclass specific antibodies (Table 1). The Tsp reactive subclass antibodies detected in the *C. trachomatis* STI cohorts showed some variation, LGT: $IgG_1$ (8.3 %), $IgG_3$ (33.3 %), UGT: $IgG_1$ (8.3 %), and Multiple: $IgG_4$ (33.3 %). 29 participants had $IgG_{1-4}$ antibodies to cHSP60. Only one of the 11 participants with serum IgG reactive against RseP was found to have serum IgG subclass reaction ($IgG_3$; Infertile cohort).

## 4. Discussion

The prevalence and type of serum IgG antibodies reactive with chlamydial proteins (HtrA, RseP, and Tsp) was examined to determine if any/ or a combination of these proteins are a significant serological marker of pathological outcomes associated with a *C. trachomatis* STI. This is the first time that Tsp and RseP have been tested as potential antigens for antibody production during *C. trachomatis* STI. Serum IgG to HtrA has been detected in previous studies although less frequently (Sharma et al., 2006). The statistical analysis of the prevalence of serum IgG reactions to each of the proteins across the cohorts

identified that cHSP60 is the only protein significantly associated with *C. trachomatis* STI. However, in contrast to a number of studies (Persson et al., 1999, Spandorfer et al., 1999), serum IgG to cHSP60 was not significantly associated with pathological outcome. This is likely due to testing for prevalence rather than titres. The participants in the Infertile cohort (negative) all tested negative by a commercial serology test for *C. trachomatis*. Nonetheless, previous undiagnosed *C. trachomatis* infections cannot be discounted. The other three cohorts all have a known *C. trachomatis* STI status based on PCR or serological based diagnoses. There is a risk that some participants in the LGT single infection cohort have actually had additional undiagnosed *Chlamydia* infections which cannot be accounted for and should be acknowledged as a potential flaw within the cohorts.

den Hartog and co-workers found that the presence or absence of serum IgG to *C. pneumoniae* did not alter the predictive value of *C. trachomatis* serological tests (den Hartog et al., 2006); and many published studies to examine serum IgG as a predictor of UGT pathology have not tested for *C. pneumoniae*. However, given this study is focussed on some novel antigens, *C. pneumoniae* serological testing was conducted. This confirmed a high prevalence of *C. pneumoniae* serum IgG within all of the cohorts. The similar frequencies of serum IgG reactions for the three antigens (HtrA, Tsp and RseP) between all of the cohorts including the negative (infertile) cohort suggests that the recombinant proteins used here are able to be detected by antibodies raised against both *C. pneumoniae* infection and *C. trachomatis* infection. Thus the impact of antibody cross reactivity is not able to be accounted for in this study.

Less than half of the participants with serum IgG for these antigens had detectable serum $IgG_{1\text{-}4}$, thus the other subclasses which were not included in this study could be important contributors. However, to our knowledge, this is the first report of serum IgG subclass testing for *C. trachomatis* using human samples. The antigens were detected by

quite distinct IgG antibody subclasses independent of disease status, with only Tsp showing a slight trend for a differential subclass reaction between disease cohorts ($IgG_4$ for M and $IgG_3$ for LGT). If we assume that different types of immune responses were occurring in the different disease cohorts when the antibody production occurred, then the results of this study suggests that for HtrA, Tsp, and RseP antigens the B-cell epitopes present on the protein were responsible for the antibody subclass production rather than the immune status. To our knowledge this is the first time that the possibility that antibody subclass reactions to *C. trachomatis* proteins may differentiate between disease cohorts has been suggested in the literature.

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## References


DEBATTISTA, J., TIMMS P, J., A. & A., A. J. (2002) Reduced levels of gamma-interferon secretion in response to chlamydial 60 kDa heat shock protein amongst women with pelvic inflammatory disease and a history of repeated *Chlamydia trachomatis* infections. *Immunol Lett,* 81**,** 205-210.

DEN HARTOG, J. E., MORRE, S. A. & LAND, J. A. (2006) *Chlamydia trachomatis*-associated tubal factor subfertility: immunogenetic aspects and serological screening. *Human Reprod Up,* 12**,** 719-730.

HUSTON, W. M., SWEDBERG, J. E., HARRIS, J. M., WALSH, T. P., MATHEWS, S. A. & TIMMS, P. (2007) The temperature activated HtrA protease from pathogen *Chlamydia trachomatis* acts as both a chaperone and protease at 37$^{o}$C. *FEBS Lett,* 581**,** 3382-3386.

HUSTON, W. M., THEODOROPOULOS, C., MATHEWS, S. A. & TIMMS, P. (2008) *Chlamydia trachomatis* responds to heat shock, penicillin induced persistence, and IFN-gamma persistence by altering levels of the extracytoplasmic stress response protease HtrA. *BMC Microbiol,* 8.

LAD, S. P., LI, J., CORREIA, J. D. S., PAN, Q., GADWAL, S., ULEVITCH, R. J. & LI, E. (2007) Cleavage of p65/Re1A of the NF-kappa B pathway by *Chlamydia*. *PNAS,* 104**,** 2933-2938.

PERSSON, K., OSSER, S., BIRKELUND, S., CHRISTIANSEN, G. & BRADE, H. (1999) Antibodies to *Chlamydia trachomatis* heat shock proteins in women with tubal factor infertility are associated with prior infection by *C. trachomatis* but not by *C. pneumoniae*. *Human Reprod,* 14**,** 1969-1973.

SHARMA, J., ZHONG, Y., FONG, F., PIPER, J. M., WANG, G. & ZHONG, G. (2006) Profiling of human antibody responses to *Chlamydia trachomatis* urogenitcal tract infection using micropaltes arrayed with 156 chlamdydial fusion proteins. *Infect Immun,* 74**,** 1490-1499.

SPANDORFER, S. D., NEUER, A., LAVERDA, D., G, B., C., L. H., Z., R. & WITKIN, S. S. (1999) Previously undetected *Chlamydia trachomatis* infection, immunity to heat shock proteins and tubal occlusion in women undergoing in-vitro fertilisation. *Human Reprod,* 14**,** 60-64.

**Table 1: Summary of results for all participants, including serum IgG antibodies, and serum $IgG_{1-4}$ antibodies reactive with HtrA, RseP, Tsp and cHSP60.**

| **Prevalence of serum IgG response detected to each antigen** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Antigen** | **LGT (n=106) (single infection)** | | **UGT (n=9) (pathology)** | | **Multiple (n=17)** | | **Infertile (n= 20)** | |
| | *C. trachomatis* positive | | | | | | *C. trachomatis* negative | |
| | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** |
| **HtrA** | 45 | 42.5 | 3 | 33.3 | 7 | 41.2 | 3 | 15 |
| **Tsp** | 16 | 15.1 | 1 | 11.1 | 4 | 23.5 | 3 | 15 |
| **cHsp60** | 69 | 65.1 | 6 | 66.6 | 10 | 58.8 | 4 | 20 |
| **RseP** | 8 | 7.6 | 0 | 0 | 1 | 5.7 | 2 | 10 |

| **Serum IgG subclass reactions** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Antigen** | **LGT (single infection)** | | | | **UGT (pathology)** | | | | **Multiple** | | | | **Infertile** | | | |
| **$IgG_{(1-4)}$ subclass** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** |
| **HtrA** | 1 | | 8 | | | 1 | 1 | | | | 1 | | 2 | 1 | | |
| **Tsp** | 1 | | 4 | | 1 | | | | | | | 4 | | 1 | | 1 |
| **cHSP60** | 1 | 1 | 16 | 1 | 1 | | 2 | | | 1 | 6 | | 6 | | | |
| **RseP** | | | | | | | | | | | | | | | 1 | |

The data is grouped according to the cohorts (see Materials and Methods for cohort definitions). Data is displayed as number of positive reactions (n) and also as percentage of participants within that cohort (%). The number of participants with serum antibodies in each $IgG_{(1-4)}$ subclass detected to react with the antigens is also shown.

**Supplementary Data**

**Click here to download Online publication only: Supplementary Data 1.docx**